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A synthetic random basic copolymer with promiscuous binding to class II major histocompatibility complex molecules inhibits T-cell proliferative responses to major and minor histocompatibility antigens *in vitro* and confers the capacity to prevent murine graft-versus-host disease *in vivo*

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ABSTRACT **Graft-versus-host disease (GVHD) is a T-cell-mediated disease of transplanted donor T cells recognizing host alloantigens. Data presented in this report show, to our knowledge, for the first time that a synthetic copolymer of the amino acids L-Glu, L-Lys, L-Ala, and L-Tyr (molecular ratio, 1.9:6.0:4.7:1.0; M_r, 6000–85000), termed GLAT, with promiscuous binding to multiple major histocompatibility complex class II alleles is capable of preventing lethal GVHD in the B10.D2 → BALB/c model (both H-2^d) across minor histocompatibility barriers. Administration of GLAT over a limited time after transplant significantly reduced the incidence, onset, and severity of disease. GLAT also improved long-term survival from lethal GVHD: 14/25 (56%) of experimental mice survived >140 days after transplant compared to 2/26 of saline-treated or to 1/10 of hen egg lysozyme-treated control mice ($P < 0.01$). Long-term survivors were documented to be fully chimeric by PCR analysis of a polymorphic microsatellite region in the interleukin 1β gene. *In vitro*, GLAT inhibited the mixed lymphocyte culture in a dose-dependent fashion across a variety of major barriers tested. Furthermore, GLAT inhibited the response of nylon wool-enriched T cells to syngeneic antigen-presenting cells presenting minor histocompatibility antigens. Prepulsing of the antigen-presenting cells with GLAT reduced the proliferative response, suggesting that GLAT inhibits antigen presentation.**

Graft-versus-host disease (GVHD) represents the major limitation to successful allogeneic bone marrow transplantation (1, 2). When the donor and recipient are major histocompatibility complex (MHC)-identical, donor T cells recognize multiple minor histocompatibility antigens (mHAg) as "nonself" (2, 3). These mHAg display a tissue-specific distribution (4). Recent experimental evidence has clearly demonstrated that human (1, 5) and murine (6–8) mHAg correspond to recipient self-peptides presented by MHC class I and II molecules. This initial recognition process of host alloantigen after transplantation is further enhanced by the release of cytokines (9, 10), resulting in nonspecific T-cell activation and in the upregulation and *de novo* expression of MHC molecules. Novel experimental approaches toward prevention of murine GVHD have focused on the molecular basis of antigen recognition by T cells and on the early stages of T-cell activation. These approaches aim at interference with the antigen-specific signal generated through the T-cell receptor (11, 12) and at the inhibition of costimulatory signals (13). We previously (11) demonstrated that small, synthetic peptides 11–14 amino acids long with high binding affinity for specific class II MHC molecules are capable of preventing murine GVHD. However, this approach has been limited by the need for allele specificity of the inhibitor peptides (11) and by the difficulty of achieving sustained tissue levels of such low molecular weight peptides over a prolonged period of time after injection (11, 14, 15).

We were intrigued by the possibility that the above two limitations might be overcome by the use of larger synthetic polypeptides with promiscuous binding to MHC class II molecules. We have previously (16) investigated a synthetic amino acid copolymer denoted Cop 1. Cop 1 has been demonstrated to be effective in suppressing experimental allergic encephalomyelitis (EAE), a T-cell-mediated autoimmune disease, in various animal species, including primates (16–18) and to be effective in reducing the relapse rate from multiple sclerosis in humans (19, 20). Subsequent studies have demonstrated the direct binding of Cop 1 to murine and human class II MHC molecules of different haplotypes (21). This interaction may result in competition with myelin antigens for activation of specific T-cell responses (22, 23). Recent studies demonstrated that Cop 1 binds directly to class II molecules without the need for intracellular processing (24). In addition, it was shown that Cop 1 is able to displace antigens from their restricting class II binding site (21). No data exist so far on the potential use of Cop 1 in GVHD. The aim of this study was to determine whether a synthetic copolymer denoted GLAT (L-Glu, L-Ala, L-Lys, and L-Tyr), which is prepared similarly to Cop 1, might be able to inhibit the early phases of T-cell alloantigen recognition, and whether this inhibition could lead to prevention of GVHD. The murine GVHD model of B10.D2/nSnJ → BALB/c (both H-2^d) across minor histocompatibility barriers has been described in detail (13, 25–27) and was selected because it represents a model that is very similar to MHC-matched bone marrow transplantation in humans (13). Data presented in this report demonstrate for the first time that administration of GLAT confers the capacity to prevent experimental GVHD.

MATERIALS AND METHODS

Animals. B10.D2/nSnJ (H-2^d), CBA (H-2^k), C57BL/6 (H-2^b), and PL/J (H-2^u) mice were purchased from The Jackson

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Abbreviations: APC, antigen-presenting cell; GLAT, a random basic synthetic copolymer of L-Glu, L-Ala, L-Lys, and L-Tyr; GVHD, graft-versus-host disease; HEL, hen egg lysozyme; MHC, major histocompatibility complex; mHAg, minor histocompatibility antigen(s); EAE, experimental allergic encephalomyelitis; IL, interleukin.

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Laboratories. BALB/c (H-2^d) recipient mice were obtained from Simonsen Laboratories (Gilroy, CA). B10.S (H-2^s) were obtained from H. O. McDevitt's colony and bred in the animal facility of Stanford University.

Bone Marrow Transplant. For the induction of GVHD, 10×10^6 bone marrow and 100×10^6 spleen cells from B10.D2 mice were injected into lethally irradiated (8.0 Gy) BALB/c recipients. This regimen induces the most severe form of GVHD (13) and was selected for all experiments of this study. Recipient mice were 12–13 weeks old at the time of transplant. All experiments were performed according to federal and Stanford University guidelines.

Copolymer and Control. The copolymer GLAT is a synthetic random basic polymer, prepared like Cop 1, by polymerization of the N-carboxyanhydrides of γ-benzyl-L-Glu, *N*,ε-trifluoroacetyl-L-Lys, L-Ala, and L-Tyr in a residue molar ratio of 1.9:6.0:4.7:1.0, followed by the removal of the blocking groups γ-benzyl and trifluoroacetyl, as previously described for Cop 1 (17, 18, 21). Two batches of GLAT were used throughout these studies with average $M_r \approx 6000–8500$. These batches were not tested in the EAE system or characterized pharmacologically as Cop 1. Therefore, they are referred to as GLAT. Hen egg lysozyme (HEL) was obtained from Sigma and was used as a negative control.

Treatment. Recipient BALB/c mice were treated with the synthetic copolymer GLAT, with phosphate-buffered saline (PBS), or with HEL. Based on previous studies with Cop 1 in EAE (16–18) and class II binding competitor peptides in GVHD (11), the dose of 600 μg per injection was selected, half of which was administered i.p., whereas the other half was given s.c. Treatment of BALB/c recipient mice was initiated on day −1, followed by daily injections for the first 5 weeks starting on day 0 after transplant. The frequency of injections was tapered to three times per week for the subsequent 2 weeks and to two times per week for another 2 weeks and then discontinued. Throughout the experiment, copolymers and controls were administered once a week with incomplete Freund's adjuvant i.p. as a depot dose (11). Treatment was discontinued 9 weeks after transplant.

Assessment of GVHD. Mice were followed daily for 140 days after bone marrow transplant for signs of GVHD. Disease severity was assessed by the following parameters: mortality, loss of body weight, and extent of macroscopic skin involvement scored on a cumulative severity scale from 0 (minimum) to 8 (maximum): head 1, neck 1, back (1/3, 2/3, 3/3) 1–3, and front (1/3, 2/3, 3/3) 1–3, as described (13, 25–27).

PCR Analysis. Engraftment of donor bone marrow was documented by PCR analysis of a polymorphic microsatellite region within the murine interleukin (IL)-1β gene. Primer sequences are as follows (13): 5′-CCAAGCTTCCTTGTGCAAGTA-3′ and 5′-AAGCCCAAAGTCCATCAGTGG-3′ (GenBank accession numbers X78456 and X78457). DNA was prepared from peripheral blood mononuclear cells 100–120 days after transplant according to standard protocols (11). PCR conditions were as follows: 25 μl total volume with 200 ng genomic DNA as template, 25 pmol primers, 0.4 mM each dNTP, 10 mM Tris·HCl (pH 8.3), 50 mM KCl, 1 mM $MgCl_2$, and 1.0 unit AmpliTAQ DNA-polymerase (Perkin–Elmer). Amplification was performed for 30 cycles: 1 min denaturation at 94°C, 1 min annealing at 57°C, and 1 min elongation at 72°C.

Mixed Lymphocyte Cultures. Mixed lymphocyte cultures were set up as described (13, 28). Briefly, 2.5×10^5 responder spleen cells were plated in flat-bottom 96-well microculture plates with 2.5, 5.0, or 10×10^5 irradiated (30 Gy, Cs source) spleen stimulator cells in a final volume of 200 μl. GLAT or relevant controls were added at the indicated concentrations (10–100 μg per well). After 96 h of incubation, cultures were pulsed with 1 μCi [^{3}H]thymidine (1 Ci = 37 GBq) for an additional 16 h. Results are mean cpm from triplicate cultures.

Inhibition of mHAg Presentation. B10.D2 mice were injected i.p. with irradiated (20 Gy) BALB/c spleen cells 72 h before the assay. B10.D2 spleen cells were harvested, depleted of erythrocytes, and used as irradiated (10 Gy) antigen-presenting cells (APC)-presenting BALB/c mHAg. APC were plated at 2.5×10^5 cells per well in 96-well round-bottom plates and incubated with 2.5×10^5 nylon wool-enriched responder T cells (>88% $CD3^+$ by fluorescence-activated cell sorter analysis) from naive B10.D2 mice in the presence or absence of increasing concentrations of GLAT (2.5–80 μg per well). After 96 h of incubation, cells were pulsed with 1 μCi [^{3}H]thymidine for an additional 16 h before harvesting. In a second experiment, irradiated APC were plated at 2.5×10^5 cells per well in 96-well round-bottom plates, preincubated for 24 h at 37°C with increasing concentrations of GLAT (2.5–80 μg per well) or with medium alone, and thereafter washed three times before the addition of 2.5×10^5 nylon wool-enriched responder T cells from naive B10.D2 mice. Culture medium was RPMI 1640, supplemented with 10% prescreened fetal calf serum, 2 mM glutamine, 50 μM 2-mercaptoethanol, 100 units of penicillin per ml, and 100 μg of streptomycin per ml.

Apoptosis. B10.D2 spleen cells (1×10^6 per ml) were cultured in 24-well plates for 48 or 72 h in the presence or absence of GLAT or HEL at the indicated concentrations. Irradiated (6.0 Gy) B10.D2 thymocytes cultured for 24 h served as the positive control (29). Cells were collected, lysed (1 mM EDTA/50 mM Tris, pH 7.5/0.1% Nonidet P-40) with 0.5 mg of proteinase K. DNA was extracted with phenol-chloroform two times and precipitated with ethanol (11). Aliquots of DNA were transferred to a 1.5% agarose gel containing 0.05 μg of ethidium bromide per ml. The presence or absence of DNA fragmentation was assessed as described (30).

RESULTS

Inhibition of MLC Across Major Histocompatibility Barriers. We tested the effect of the synthetic copolymer GLAT on mixed lymphocyte cultures across major histocompatibility barriers. T-cell proliferation was assessed in six different MHC-disparate strain combinations. Data given in Fig. 1 are representative of five separate experiments with similar results. In all experiments, addition of GLAT (10–100 μg per well) resulted in a dose-dependent inhibition of the MLC. HEL showed no or only a minimal inhibitory effect at all concentrations tested (Fig. 1). GLAT at 10–25 μg per well was sufficient to achieve 50% inhibition of the proliferative responses. Maximum inhibition (100%) was obtained in all strain combinations tested. To exclude the possibility that higher concentrations of GLAT (50–100 μg per well) might be toxic to the responder cells, responder cells from background wells and from wells incubated with 25–100 μg per well of GLAT in the presence of stimulator cells for 72 h were rechallenged in a subsequent assay with IL-2. As shown in Table 1, there was no difference between the secondary proliferative responses of the groups tested. Responder cells that had been incubated with stimulator cells in the presence of GLAT for 72 h were equally responsive to IL-2, compared to nontreated cells.

Inhibition of Proliferative Responses to mHAg *in Vitro*. Nylon wool-enriched T cells (>88% $CD3^+$ cells by fluorescence-activated cell sorter analysis) from naive B10.D2 were incubated for 96 h with syngeneic APC-presenting minor BALB/c antigens as described in *Materials and Methods*. As shown in Table 2, GLAT inhibited the proliferative responses to mHAg in a dose-dependent fashion. Maximum inhibition was 100% at a concentration of 80 μg per well. To further elucidate the mechanism of inhibition, APC were preincubated for 24 h with increasing concentrations of GLAT (2.5–80 μg per well), washed, and plated thereafter with responder T

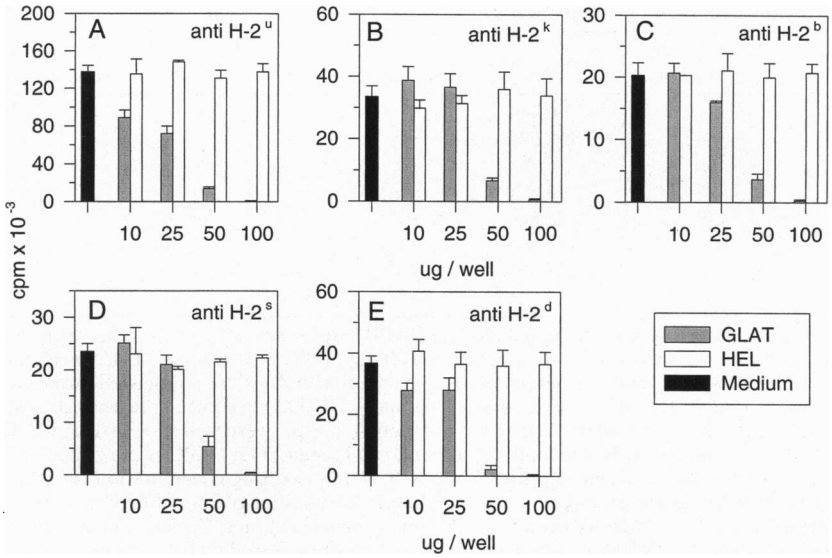

FIG. 1. Dose-dependent inhibition of the MLC across major histocompatibility barriers (*A–D*, H-2^d anti-H-2^{u,s,k,b}; *E*, H-2^b anti-H-2^d). Responder spleen cells (2.5×10^5) were incubated with optimal concentrations of irradiated (30 Gy, Cs source) spleen stimulator cells as described in a final volume of 200 μl. GLAT or HEL were added at the indicated concentrations (10–100 μg per well). After 96 h of incubation, cultures were pulsed with 1 μCi [^{3}H]thymidine for an additional 16 h. Similar degrees of inhibition by GLAT were observed using various amounts of stimulator cells (2.5, 5.0, or 10×10^5; data not shown). No inhibition was observed with the addition of HEL in any of the strain combinations tested. Results represent one of five experiments with similar results.

cells. As shown in Table 2, preincubation with GLAT inhibited proliferative responses to mHAg, suggesting that GLAT inhibits presentation of minor antigens *in vitro*.

Absence of Apoptosis. Ligation of MHC class II molecules has recently been shown to mediate apoptotic cell death in resting B lymphocytes (31). To determine whether GLAT might induce apoptosis in class II bearing cells (B cells and/or T cells), murine spleen cells were incubated with increasing concentrations of GLAT or HEL control for 48 or 72 h. Aliquots of cells were processed for DNA extraction and DNA was visualized by gel electrophoresis. As shown in Fig. 2, GLAT did not induce apoptosis at any of the concentrations tested.

Effect of GLAT Treatment on the Incidence, Onset, and Severity of GVHD. After demonstrating that the synthetic copolymer GLAT was able to inhibit T-cell proliferative responses to both major and minor histocompatibility antigens *in vitro*, we investigated whether administration of GLAT could lead to prevention of GVHD *in vivo*. Recipient mice were pretreated with 600 μg of GLAT or with the respective controls (PBS or HEL) on day −1. For the first 5 weeks after transplant, mice were injected daily as outlined in *Materials and Methods*, followed by a tapering schedule over an additional 4 weeks. Data from three consecutive experiments are summarized in Fig. 3. Administration of GLAT significantly reduced the overall incidence of GVHD (as determined by typical skin changes and weight loss) from 100% (26/26, 10/10) in control mice to 12% (3/25) ($P < 0.001$) in GLAT-treated animals on day 30 after transplant, and from 100% in controls to 12/25 (48%) on day 70 after transplant ($P < 0.02$). Fig. 3*A* depicts the onset of GVHD in individual mice of the different experimental groups. In 12/25 animals treated with GLAT, the onset of GVHD was delayed with a range of 32–112 days after transplant (median of 73 days) as compared to control mice treated with either PBS (median onset, 21 days) or HEL (median onset, 22 days). Nine of 25 animals treated with the copolymer GLAT did not develop any signs of GVHD beyond the observation period of 140 days after transplant (Fig. 3*A*). Furthermore, treatment with GLAT decreased overall disease severity as gauged by the disease severity score (Fig. 3*B*) and by mean body weight curves of transplanted animals (Fig. 3*D*).

Table 1. Effect of incubation with different concentrations of GLAT on the subsequent proliferative response to restimulation of IL-2

Primary responder	Assay stimulator	GLAT, μg per well	cpm	Secondary Stimulus	Assay cpm
B10.D2		–	564	IL-2	9,170
B10.D2	C57BL/6	–	20,333	IL-2	8,305
B10.D2	C57BL/6	100	283	IL-2	7,951
B10.D2	C57BL/6	50	3,663	IL-2	15,123
B10.D2	C57BL/6	25	15,953	IL-2	11,994

Primary assay (left): 2.5×10^5 responder spleen cells were incubated with 5×10^5 irradiated (30 Gy) spleen stimulator cells in a final volume of 200 μl in the presence or absence of GLAT as indicated. After 96 h of incubation, cultures were pulsed with 1 μCi [^{3}H]thymidine for an additional 16 h. Secondary assay (right): identical cultures were set up in parallel to the primary assay. After 72 h of incubation, responder cells were rechallenged with IL-2 (5000 units/well) for an additional 48 h before pulsing with 1 μCi [^{3}H]thymidine for an additional 16 h. Results are expressed as mean cpm for triplicate cultures. Standard deviations were <16% of the mean.

Effect of GLAT Treatment on Survival. Treatment with GLAT improved long-term survival from lethal GVHD. As shown in Fig. 3*C*, 14/25 (56%) of experimental mice survived >140 days after transplant as compared to 2/26 of PBS-treated mice or to 1/10 of HEL-treated control mice ($P < 0.01$). Treatment with HEL did not improve long-term survival.

Documentation of Complete Chimerism. PCR analysis was performed to document long-term engraftment of allogeneic bone marrow cells. DNA polymorphism based on length variation in tandem repeat sequences of a microsatellite in the murine IL-1β gene was used as marker to differentiate between donor-derived (B10.D2/nSnJ) and recipient (BALB/c) peripheral blood mononuclear cells. Complete chimerism was demonstrated in all allogeneic mice irrespective of the treatment received. Fig. 4 shows one representative analysis.

DISCUSSION

GVHD still represents the major barrier to successful allogeneic transplantation (1). Negative and positive selection stud-

Table 2. Dose-dependent inhibition of proliferative responses to mHAg

Responder	APC	GLAT assay concentration, μg	cpm	Responder	APC	Prepulsed APC	GLAT pulsing concentration, μg	cpm
+	−	−	10,464	+	−	−	−	7,855
+	+	−	108,574	+	+	−	−	16,724
+	+	80	361	+	−	+	80	4,628
+	+	40	23,591	+	−	+	40	7,653
+	+	20	75,840	+	−	+	20	10,465
+	+	10	119,866	+	−	+	10	11,425
+	+	5	126,405	+	−	+	5	12,377
+	+	2.5	124,904	+	−	+	2.5	15,533

Left: B10.D2 mice were injected i.p. with irradiated (20 Gy) BALB/c spleen cells 72 h before the assay. B10.D2 spleen cells were harvested, depleted of erythrocytes, and used as irradiated (10 Gy) APC-presenting BALB/c mHAg. APCs were plated at 2.5×10^5 cells per well in 96-well round-bottom plates and incubated with 2.5×10^5 nylon wool-enriched responder T cells (>88% CD^{3+} by fluorescent-activated cell sorter analysis) from naive B10.D2 mice in the presence or absence of increasing concentrations of GLAT (2.5–80 μg per well). After 96 h of incubation, cells were pulsed with 1 μCi [^{3}H]thymidine for an additional 16 h before harvesting. Right: In a second experiment, irradiated (20 Gy) APCs were plated at 2.5×10^5 cells per well in 96-well round-bottom plates, preincubated for 24 h at 37°C with increasing concentrations of GLAT (2.5–80 μg per well) or with medium alone, and thereafter washed three times before the addition of 2.5×10^5 nylon wool-enriched responder T cells from naive B10.D2 mice. Results are expressed as cpm from triplicate cultures. Typical standard deviations were <20% of the mean. Culture medium was RPMI 1640, supplemented with 10% prescreened fetal calf serum, 2 mM glutamine, 50 μM 2-mercaptoethanol, 100 units of penicillin per ml, and 100 μg of streptomycin per ml.

ies have unequivocally demonstrated that donor T cells are responsible for the induction phase of GVHD in both murine and human systems (26, 27, 32). Activated T cells proliferate, secrete cytokines, recruit additional inflammatory cells (25), and lead to the effector phase of GVHD through multiple primary and secondary mechanisms (32). Recent research has focused on the early cellular and molecular events involved in antigen recognition in GVHD (11–13). The aim of this study was to investigate whether a novel class of biological response modifiers with promiscuous binding to multiple class II MHC alleles could inhibit host alloantigen recognition and thus prevent GVHD.

GVHD is an alloimmune response induced by transplanted donor T cells in response to multiple minor antigens of the recipient (32). Some of the mHAg may occupy up to 10% of binding sites, whereas others may occupy as little as 0.001% of MHC sites (33). Our previous work (11) has demonstrated that GVHD can be prevented through the use of class II binding allele-specific peptides of 11–14 amino acids. Prevention was found to be transient in some and permanent in others (11). However, this approach has been limited by the need for allele-specificity (11) and by the relatively short half-life of class II peptides (11, 14, 15). GLAT, being of larger molecular weight, is expected to have an extended half-life, in addition to its promiscuous but specific binding properties for multiple MHC class II alleles. The above considerations provided the rationale for the design of the studies presented in this report.

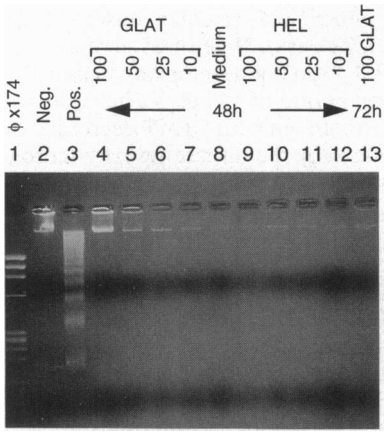

FIG. 2. Detection of apoptosis. B10.D2 spleen cells were cultured for 48 h (lanes 4–12) or 72 h (lane 13) in the presence or absence of GLAT or HEL at the indicated concentrations (10–100 μg/200 μl). Irradiated (6.0 Gy) B10.D2 thymocytes cultured for 24 h (30) served as the positive control (lane 3); spleen cells from naive B10.D2 mice (lane 2) or spleen cells cultured in medium for 48 h (lane 8) were used as negative controls. Lane 1: DNA marker ϕX174 digested with *Hae* III. Cells were collected and lysed as described. DNA was extracted with phenol-chloroform and precipitated with ethanol (11). Aliquots of DNA were transferred to a 1.5% agarose gel containing 0.05 μg ethidium bromide per ml. The presence or absence of DNA fragmentation was assessed as described (30).

A series of *in vitro* experiments using T-cell proliferative responses to major and minor histocompatibility antigens were performed. As shown in Fig. 1, addition of GLAT inhibited the mixed lymphocyte cultures in a dose-dependent inhibition in a variety of systems (H-2^d anti H-$2^{u,s,k,b}$ or H-2^b anti H-2^d). HEL was used as specificity control. To exclude any toxic effect of GLAT on the responder cells, cells were successfully rechallenged with IL-2 in a subsequent assay (Table 1). Furthermore, GLAT (1–100 μg per well) did not inhibit the IL-2-driven proliferation of the murine T-cell clone G81 (data not shown). To investigate the role of T-cell responses to minor antigens, a system was designed that allowed for antigen presentation of mHAg on syngeneic APC (Table 2). GLAT reduced the proliferative responses to mHAg in a dose-dependent fashion. Maximum inhibition was found at a concentration of 80 μg per well. In an additional experiment, APC were harvested, preincubated for 24 h with different concentrations of GLAT, and washed before incubation with the responder cells. Prepulsing of the APC with GLAT reduced the subsequent proliferative response to mHAg (Table 2), suggesting that GLAT inhibits antigen presentation.

Ligation of MHC class II molecules has recently been shown to mediate apoptotic cell death in resting B lymphocytes under certain conditions (31). As shown in Fig. 2, incubation of spleen cells with GLAT for 48 or 72 h did not result in apoptosis. These results were confirmed by fluorescence-activating cell sorter analysis using Hoechst dye 33342 (data not shown).

Data from three consecutive experiments *in vivo* showed that administration of GLAT over a limited period after transplant prevented GVHD across minor histocompatibility barriers. Nine of 25 animals did not develop GVHD beyond the observation period of 140 days posttransplant, whereas all control-treated mice (PBS or HEL) had developed GVHD by day 26 (Fig. 3*A*). Furthermore, 12 of the GLAT-treated mice showed a delayed onset of GVHD (range, 32–112). GLAT decreased disease severity as assessed by the severity score (Fig. 3*B*) and by the mean body weight curves (Fig. 3*D*). GLAT

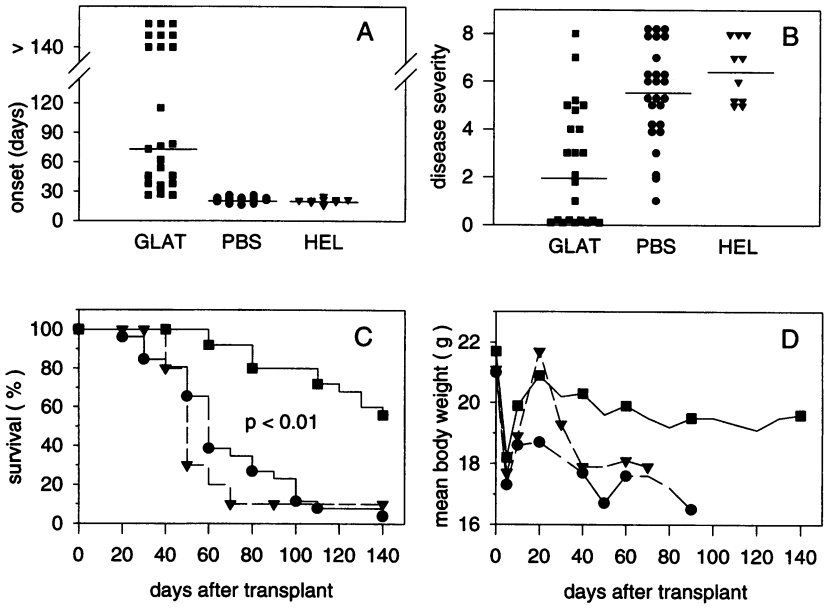

FIG. 3. Effect of treatment with GLAT on the induction of GVHD in B10.D2/nSnJ → BALB/c recipients (■, GLAT, $n = 25$; ●, PBS, $n = 26$; ▼, HEL, $n = 10$). (*A*) Onset of GVHD in individual mice of the experimental groups. The median disease onset is indicated by lines (day 73 for GLAT, day 21 for PBS, and day 22 for HEL). Statistical analysis by χ^2 distribution. Day 30 after transplant: $P < 0.001$, incidence of GVHD in GLAT-treated mice (3/25) compared to the incidence in mice treated with PBS (26/26) or with HEL (10/10). Day 70 after transplant: $P < 0.001$, incidence of GVHD in GLAT-treated mice (12/25) compared to PBS (26/26), or $P < 0.02$ compared to HEL (10/10). (*B*) Maximum disease severity in individual mice of the experimental groups. The median disease severity is indicated by lines (2.0 for GLAT, 5.5 for PBS, 6.5 for HEL). (*C*) Actuarial survival from lethal GVHD. The median survival times (days) for the experimental groups are as follows: >140 days for GLAT, 47 days for PBS, and 41 days for HEL. Statistical analysis by Mann–Whitney test: $P < 0.01$, GLAT-treated mice compared to control mice treated with either PBS or HEL. (*D*) Mean body weight curves after transplant. Data in *A–D* are pooled from three similar experiments.

resulted in a significantly improved survival from GVHD-related mortality ($P < 0.01$) as compared to controls (Fig. 3*C*). The selected system of GVHD closely resembles the clinical setting of matched donor-recipient pairs (13, 32). The murine system of B10.D2/nSnJ → BALB/c has been well elaborated (3, 13, 25–27). Both $CD4^+$ and $CD8^+$ T-cell subsets are responsible for GVHD induction in lethally irradiated BALB/c recipients, with $CD4^+$ T cells being the predominant subset (27).

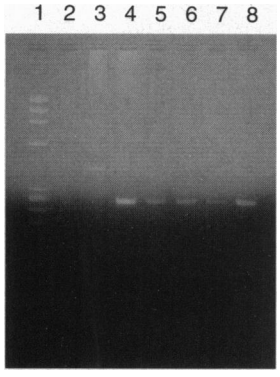

FIG. 4. Documentation of complete chimerism. Engraftment of donor bone marrow was demonstrated by PCR amplification of a polymorphic microsatellite region within the murine IL-1β gene. Primers and reaction conditions are given in *Materials and Methods*. DNA was prepared from peripheral blood mononuclear cells 100–120 days after transplant according to standard protocols (11). All transplanted mice were found to be chimeras irrespective of which therapy they had received. This figure depicts one representative analysis on an ethidium bromide-stained 2% agarose gel. Lanes: 1, DNA marker φX174 digested with *Hae* III (marker sizes: 1353, 1078, 872, 603, 310, 281, 271, and 234 bp; GIBCO/BRL); 2, blank; 3, recipient (BALB/c) standard; 4, donor (B10.D2) standard; 5 and 6, GLAT-treated mice without GVHD (ID 477 and 478); 7, HEL-treated mouse with GVHD (ID 489); 8, PBS-treated mouse with GVHD (ID 498).

GLAT is a synthetic random basic copolymer prepared similarly to Cop 1. Previous work on the suppression of EAE and multiple sclerosis has demonstrated two main and distinct mechanisms of action for Cop 1, which derive from its direct high-affinity binding to class II molecules of multiple murine and human alleles (21): (*i*) cross-reactivity with myelin basic protein at the humoral (34) and cellular (35) level leading to the induction of suppressive regulatory cells (36), and (*ii*) competitive inhibition of the binding of myelin antigens to MHC, which may result in inhibition of specific effector T-cell activation (22, 23). The first mechanism has been shown to be most important for the prevention of EAE (36), a model in which a dominant antigen (myelin basic protein for $H\text{-}2^u$ and proteolipid protein for $H\text{-}2^s$ strains) leads to the induction phase of the autoimmune disease. On the contrary, GVHD is an alloimmune response to multiple minor antigens of the recipient (32). The relationship between the activities of Cop 1 in EAE and GLAT in GVHD is not clear and remains to be determined.

The results of the *in vitro* and *in vivo* studies described in the present study are consistent with the concept that GLAT by virtue of its ability to bind to multiple MHC class II molecules inhibits antigen presentation. Administration of GLAT over a limited time after transplant was successful in preventing the development of GVHD. The effects of GLAT on GVHD induced by class I or class II disparities, or on the graft-versus-leukemia reactivity, will be addressed in future studies. Data presented in this report show for the first time that GLAT represents a novel class of potent biological response modifiers for the prevention of GVHD across minor histocompatibility barriers.

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